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Dear Herman:

How go the Gal$^-$ mutants, human and coliform? More precisely, have you had any luck so far with the galactose system of the Gal$^+$ strain?

I may have been too vague on the suggested protocol for growing the organisms. For preliminary purposes, it would be simplest to grow the cells directly on a galactose medium; the following composition would do as well as any: (per liter) galactose 2gms, K_2HPO_4 7, KH_2PO_4 2; Na_3 citrate.$5H_2O$ 0.5; $MgSO_4.7H_2O$ 0.1; $(NH_4)_2SO_4$ 1. It might be best to sterilize the galactose separately as a 20% solution, either by filtration or autoclave as you judge best, and add to the rest of the medium. The cultures can be maintained on ordinary nutrient agar slants, but for inoculating a batch I would grow first on the above medium (Davis') with glucose instead of galactose. The cultures should be aerated vigorously, most conveniently by shaking or rotation; for larger batches you may have to bubble air. They will grow conveniently at almost any temperature, 30-37° C. probably best.

When the Gal+ has been successfully handled this way, then I suggest you run it through the following modification: grow a mass of cells on Davis' medium with glycerol or sodium succinate as the carbon source. Then dilute the cells in Davis' medium containing <u>both</u> glycerol and galactose. The cells need be diluted only about 2-fold. Culture for 2 to three hours <u>only</u>! Then harvest and assay. The point of this procedure is to avert the selection of Gal+ revsrions which will occur rarely in some of the mutants. The Gal$^-$ stocks cannot use galactose as carbon source anyhow, so there is no point testing them after trying to grow them on a strictly galactose medium. You can't use glucose as the accessory carbon source as this will prevent adaptation to galactose.

Another important question that has never been studied is the utilizability of some of the intermediates by intact cells; of course, you will have to use cells that have previously been adapted (i.e. exposed) to galactose.

During our very enjoyable visit, you mentioned somethingabout increasing the permeability of E. coli to ATP by exposure to BuOH. I have forgotten the details— can you remind me of them, or give me a reference?

If you have had any problems, or if I can be of any help at all, please let me know.

Yours